Bioaccumulation and biotransformation of pharmaceuticals in zebrafish (*Danio rerio*) eleutheroembryos by GC-MS and LC-MS/MS.

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In the last decades the consumption of human and animal drugs has raised dramatically. As a consequence, some pharmaceuticals and their residues have been detected in surface waters, mainly in the effluvium of wastewater treatment plants. Presence of these compounds could have adverse effects on aquatic organisms. For this reason pharmaceuticals are currently considered as potential emergent contaminants [1].

So, the environmental impact of pharmaceutical has become a major concern for the public, as well as for regulators and for scientists. The European Legislation (REACH) requires the evaluation of the ecotoxicity of commercialized chemicals as well as information on their bioaccumulation factors (BCF) or persistence. OECD Guideline 305 describes methods to determine the BCF using adult fish. However, the methodology proposed is complex and costly (more than one hundred animals are required) [2]. For this reason, with the main aims of reducing animal suffering and high cost, we propose an alternative methodology in which zebrafish eleutheroembryos are used as model organisms to calculate BCFs.

In this study, three groups of pharmaceutical products have been selected: a) non-steroidal anti-inflammatories (NSAIDs) such as ibuprofen, naproxen and diclofenac, b) lipid regulators such as clofibric acid and c) antidepressant selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, sertraline, citalopram, and paroxetine.

Zebrafish eleutheroembryos were exposed to the selected compounds at two concentrations (1% and 0.1% the LC_{50}), following the OECD 305 guideline during 48 h. Subsequently, the embryos were transferred to clean media during further 24 h to simulate a depuration process. About 8-10 mg (20 eleutheroembryos) and media samples were taken at different times to analyze the concentration of the pharmaceuticals.

To overcome the analytical problems associated to the low concentrations of the compounds in these extremely small samples, several extraction strategies (USP, vortex, several solvents, DLE, use of ionic liquids) and cleaning sorbents (C_{18}, Florisil, Z-Sep, Carbon) have been tested. Gas chromatography and liquid chromatography with mass spectrometer detectors (GC-MS and LC-MS/MS) have been used for separation/quantification.

A preliminary study, using a previously developed protocol based on toxicokinetic models [3] employing eleutheroembryos exposed at 5 and 30 µg/L to evaluate the bioconcentration factors (BCFs) has evidenced a quite low BCFs (below 5) and biotransformation of parent compounds. Results on further experiments designed to calculate BCFs and to check possible biotransformations of parent compounds and elucidate metabolism of SSRI in zebrafish eleutheroembryos will be reported.

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[1] M. Lahti, J.-M. Brozinski, H. Segner, L. Kronberg, A. Oikari, Bioavailabilityof pharmaceuticals in waters close to wastewater treatment plants: use of fish bile for exposure assessment*, Environ. Toxicol. Chem. 2012, 31, 1831–1837*.
[2] OECD (Ed.), Test No 305: Bioconcentration: Flow-through Fish Test (2012).
[3] J. Sanz-Landaluze, M. Pena-Abaurrea, R. Muñoz-Olivas, L. Ramos, C. Cámara. Alternative zebrafish (Danio rerio) eleutheroembryo-based procedure for bioaccumulation evaluation. *Environ. Sci. Technol., 2015, 49(3), 1860–1869.*